#5

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(MBHB Ref. No. 00-816-C (RPI Ref. No. 700.002))

In re Application of: Usman et al.	)	
	)	Group Art Unit: Not Assigned
Serial No.: 09/877,526	)	
	)	Examiner: Not Assigned
Filed: June 8, 2001	)	
	)	
For: NUCLEIC ACID SENSOR MOLECULES	)	

PRELIMINARY AMENDMENT AND RESPONSE TO THE NOTICE TO FILE MISSING PARTS MAILED JULY 26, 2001

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Pursuant to the Notice to File Missing Parts mailed July 26, 2001, the Applicant requests the following Declaration, Drawings, Sequence Listing, and amendments be entered prior to examination of the above-mentioned application on the merits. It is believed that no fee is due for filing this response; however, if a fee is due, the Commissioner is authorized to charge our Deposit Account No.13-2490.

AMENDMENT

In the Specification:

On page 68, please replace the paragraph beginning at line 14 with the following substitute paragraph:

"Figure 26 shows a non limiting example of target signaling molecule inactivation of a zinzyme sensor molecule. In the absence of the target (SEQ ID NO. 34), the zinzyme sensor molecule (SEQ ID NO. 35) catalyzes the cleavage of a reporter molecule (SEQ ID NO. 36)."

CERTIFICATE OF MAILING (37 C.F.R. 1.8a)

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail (37 C.F.R. 1 8a) on the date indicated below and is addressed to the: Assistant Commissioner for Patents, Washington D C 20231.

Date of Deposit: September 26, 2001

Jammey Kligis

Jammey Kligis

On page 68, please replace the paragraph beginning at line 18 with the following substitute paragraph:

"Figure 27 shows a non-limiting example of target signaling molecule activation of a zinzyme sensor molecule. In the presence of the target (SEQ ID NO. 37), the zinzyme sensor molecule (SEQ ID NO. 38) catalyzes the cleavage of a reporter molecule (SEQ ID NO. 39)."

On page 68, please replace the paragraph beginning at line 22 with the following substitute paragraph:

"Figure 28 shows a non-limiting example of a nucleic acid sensor molecule that is modulated by a protein target signaling molecule, Erk. In the presence of the target protein (Erk), the nucleic acid sensor molecule (SEQ ID NO. 41) catalyzes the cleavage of a reporter molecule."

On page 68, please replace the paragraph beginning at line 26 with the following substitute paragraph:

"**Figure 29** shows a non-limiting example of a "half-zinzyme" nucleic acid sensor molecule that is modulated by the 5'-UTR of the Hepatitis C virus (HCV 5'-UTR). The figure shows both inactive and active forms of the zinzyme sensor molecule (SEQ ID NO. 43). In the presence of the target signaling oligonucleotide (SEQ ID NO. 26) which represents the stem loop IIIB of the HCV 5'-UTR, the zinzyme sensor demonstrates an activity increase of three logs in cleaving the reporter molecule component of the sensor molecule as shown in the graph (+ oligo target) as compared to the sensor molecule in the absence of the target. In the presence of the full length 350 nt. HCV 5'-UTR, the zinzyme sensor molecule demonstrates an almost one log increase in activity in cleaving the reporter molecule component of the sensor molecule."

On page 94, please replace the paragraph beginning at line 27 with the following substitute paragraph:

"Figure 26 shows a non-limiting example of target signaling molecule inactivation of a zinzyme sensor molecule. In the absence of the target (SEQ ID NO. 34), the zinzyme sensor molecule (SEQ ID NO. 35) catalyzes the cleavage of a reporter molecule (SEQ ID NO. 36).

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
Chicago, Illinois 60606
Phone 312-913-0001
Fax: 312-913-0002

Reaction conditions: 140mM KCl, 10mM NaCl, 20 mM HEPES pH 7.4, 1mM $MgCl_2$, 1mM $CaCl_2$, 400 nM Nucleic acid sensor, 400 nM Target, Trace of labeled reporter (~10 nM), 25µl reaction volume, Nucleic acid sensor, target and reporter were heated at 75°C for 3 min, cooled to 37°C and cleavage initiated by the addition of $MgCl_2$ and $CaCl_2$."

On page 95, please replace the paragraph beginning at line 6 with the following substitute paragraph:

"Figure 27 shows a non-limiting example of target signaling molecule activation of a zinzyme sensor molecule. In the presence of the target (SEQ ID NO. 37), the zinzyme sensor molecule (SEQ ID NO. 38) catalyzes the cleavage of a reporter molecule (SEQ ID NO. 39). Reaction conditions: 140mM KCl, 10mM NaCl, 20 mM HEPES pH 7.4, 1mM $MgCl_2$, 1mM $CaCl_2$, 400 nM Nucleic acid sensor, 400 nM Target, Trace of labeled reporter (~10 nM), 25µl reaction volume, Nucleic acid sensor, target and reporter were heated at 75°C for 3 min, cooled to 37°C and cleavage initiated by the addition of $MgCl_2$ and $CaCl_2$."

On page 96, please replace the paragraph beginning at line 12 with the following substitute paragraph:

"An RNA sensor domain that binds to protein ERK2 (Erk) was appended to a variant of the hammerhead enzymatic nucleic acid molecule through a communication module developed through rational design. The salient feature of this design strategy is that substrate-binding elements in the enzymatic nucleic acid molecule domain are sequestered by complementary allosteric effector sequences present in the communication module in the absence of target. Target association with the sensor domain forces an alternative RNA conformation in which the substrate binding elements become available for interaction with cleavage substrate, thus promotion catalysis. **Figure 28** shows a non-limiting example of a nucleic acid sensor molecule that is modulated by a protein target signaling molecule, Erk. In the presence of the target protein (Erk), the nucleic acid sensor molecule (SEQ ID NO. 41) catalyzes the cleavage of a reporter molecule. Reaction conditions: 100mM KCl, 1mM $MgCl_2$, 10mM Tris 7.5, 10µM ERK protein, 1µM HH ribozyme, Vf=19µl, 34°C for 30 minutes, trace 5' labeled substrate (1µl)."

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
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On page 99, please replace the paragraph beginning at line 21 with the following substitute paragraph:

"Figure 29 shows a non-limiting example of a "half-zinzyme" nucleic acid sensor molecule with a PEG linker that is modulated by the 5'-UTR of the Hepatitis C virus (HCV 5'-UTR). The figure shows both inactive and active forms of the zinzyme sensor molecule (SEQ ID NO. 43). In the presence of the target signaling oligonucleotide (SEQ ID NO. 26) which represents the stem loop IIIB of the HCV 5'-UTR, the zinzyme sensor demonstrates an activity increase of three logs in cleaving the reporter molecule component of the sensor molecule as shown in the graph (+ oligo target) as compared to the sensor molecule in the absence of the target. In the presence of the full length 350 nt. HCV 5'-UTR, the zinzyme sensor molecule demonstrates an almost one log increase in activity in cleaving the reporter molecule component of the sensor molecule. Reaction conditions: 140mM KCl, 10mM NaCl, 20 mM HEPES pH 7.4, 1mM $MgCl_2$, 1mM $CaCl_2$, 400 nM Nucleic acid sensor, 400 nM Target, Trace of labeled reporter (~10 nM), 25µl reaction volume, Nucleic acid sensor, target and reporter were heated at 75°C for 3 min, cooled to 37°C and cleavage initiated by the addition of $MgCl_2$ and $CaCl_2$."

Please replace originally filed Table III (page 112) with amended Table III (page 112) herewith enclosed.

A marked up copy of the changes made to the originally filed specification page is attached as APPENDIX A.

In the Figures:

Please replace Figures 1 to 34 with substitute Figures 1 to 34 submitted herewith.

The Sequence Listing:

Please incorporate the sequence listing submitted herewith into the specification. The sequence listing, submitted in printed form and computer readable form in compliance with 37

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
Chicago, Illinois 60606
Phone: 312-913-0001
Fax: 312-913-0002

C.F.R. §§ 1.821-1.825, corresponds with sequences found in Table III, sequences found in Figures 1 to 34 and sequences found in the specification. No new matter has been added by this substitute sequence listing.

REMARKS

Specification

On page 68, the paragraph beginning at line 14 has been amended to incorporate SEQ ID NOS to correspond with Figure 26. On page 68, the paragraph beginning at line 18 has been amended to incorporate SEQ ID NOS to correspond with Figure 27. On page 68, the paragraph beginning at line 22 has been amended to incorporate SEQ ID NOS to correspond with Figure 28. On page 68, the paragraph beginning at line 26 has been amended to incorporate SEQ ID NOS to correspond with Figure 29. On page 94, the paragraph beginning at line 27 has been amended to incorporate SEQ ID NOS to correspond with Figure 26. On page 95, the paragraph beginning at line 6 has been amended to incorporate SEQ ID NOS to correspond with Figure 27. On page 96, the paragraph beginning at line 12 has been amended to incorporate SEQ ID NOS to correspond with Figure 28. On page 99, the paragraph beginning at line 21 has been amended to incorporate SEQ ID NOS to correspond with Figure 29. A marked-up version of the specification is submitted as Appendix A.

Substitute Table III has been amended so that the name of the sequences corresponds with the data in Figure 16.

No new matter has been added by way of these amendments to the specification.

Figures

Amendments have been made in Figures 1 to 34 to correct the margins in compliance with 37 CFR 1.84 (g) and to include the SEQ ID NOS to identify the oligonucleotides in compliance with 37 CFR §1.821-1.825. Amendments have also been made to further clarify the subject matter of the Figures.

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
Chicago, Illinois 60606
Phone. 312-913-0001
Fax: 312-913-0002

Replacement Figures 1 – 34 are submitted herewith. Marked-up versions of originally filed Figures 19, 20, 26, 27 and 29 are attached as Appendix B.

The Office has objected to the Figures for excessive text. Applicant submits that the text in Figures 1 – 34 is necessary for the understanding of the drawings and is therefore not excessive. Applicant respectfully requests withdrawal of the objection. If this objection is maintained, Applicant respectfully requests that guidance be provided as to which text is considered excessive for each figure.

No new matter has been added by way of these amendments to the Figures.

Sequence Listing

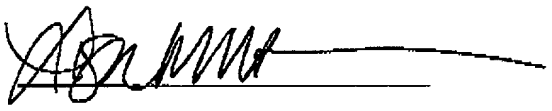
In compliance with 37 C.F.R §§ 1.821-1.825, the Applicant herewith submits the substitute Sequence Listing in written form and computer readable form (3.5 inch disk). The written and computer readable forms of the Sequence Listing are the same. The Statement under 37 CFR § 1.821(f) and § 1.825 (b) is also provided.

The Sequence Listing has been generated from the specification and does not constitute new subject matter. The Sequence Listing has been prepared with PatentIn Ver.3.0 and checked with Checker Version 3.0 Program. No error has been found.

Conclusion

If the Examiner has any questions regarding the foregoing, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,



Lisa M. W. Hillman
Registration No. 43,673

Date: September 26, 2001

McDonnell Boehnen Hulbert & Berghoff
300 South Wacker Drive
Chicago, Illinois 60606
Phone: 312-913-0001

Table III: Ribozyme effector molecule sequence

RPI#	Name	Sequence	Seq. ID No.
15404	S-2.1 & 2.7	AAGCACUAAUGGAGA	1
17161	S-3.1	AAGCACUAACAGUAA	2
15400	Rz-2.1	UCUCCAU CUGAUGAGGCCGUUAGGCCGAA AGUGCUUG	3
17159	Rz-2.7	UCUCCAU CUGAUGAGGCCGUUAGGCCGAA AGUGCUUG CGAGUG	4
17160	Rz-3.1	UUACUGU CUGAUGAGGCCGUUAGGCCGAA AGUGCUUG CGAGUG	5
17162	I-2.1	caagcacuuucucaucagauggaga	6
17163	I-2.2	cacucgcaagcacuuucucaucagauggaga	7
17164	I-2.3	cacucgcaagcacccuaucaggcagua	8
17165	I-2.4	cacucgcaagcacccuaucagguggaga	9
15405	T-2a	UACUGCCUGAUAGGGUGCUUGCGAGUG	10

UPPER CASE = RIBO
lower case = 2'-O-methyl

Appendix A

Version With Markings to Show Changes Made to Specification

Bold, Underlined Text indicates inserted text

Bracketed text indicates deleted text

In the Specification:

The paragraph beginning on Page 68, line 14:

Figure 26 shows a non limiting example of target signaling molecule inactivation of a zinzyme sensor molecule. In the absence of the target (SEQ ID NO. [31]**34**), the zinzyme sensor molecule (SEQ ID NO. [32]**35**) catalyzes the cleavage of a reporter molecule (SEQ ID NO. [33]**36**).

The paragraph beginning on Page 68, line 18:

Figure 27 shows a non-limiting example of target signaling molecule activation of a zinzyme sensor molecule. In the presence of the target (SEQ ID NO. [34]**37**), the zinzyme sensor molecule (SEQ ID NO. [35]**38**) catalyzes the cleavage of a reporter molecule (SEQ ID NO. [36]**39**).

The paragraph beginning on Page 68, line 12:

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The paragraph on Page 68, line 26:

Figure 29 shows a non-limiting example of a "half-zinzyme" nucleic acid sensor molecule that is modulated by the 5'-UTR of the Hepatitis C virus (HCV 5'-UTR). The figure shows both inactive and active forms of the zinzyme sensor molecule (SEQ ID NO. [42]**43**). In the presence of the target signaling oligonucleotide (SEQ ID NO. [43]**26**) which represents the stem loop IIIB of the HCV 5'-UTR, the zinzyme sensor

demonstrates an activity increase of three logs in cleaving the reporter molecule component of the sensor molecule as shown in the graph (+ oligo target) as compared to the sensor molecule in the absence of the target. In the presence of the full length 350 nt. HCV 5'-UTR, the zinzyme sensor molecule demonstrates an almost one log increase in activity in cleaving the reporter molecule component of the sensor molecule.

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The paragraph beginning on Page 95, line 6:

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The paragraph beginning on Page 96, line 12:

An RNA sensor domain that binds to protein ERK2 (Erk) was appended to a variant of the hammerhead enzymatic nucleic acid molecule through a communication module developed through rational design. The salient feature of this design strategy is that substrate-binding elements in the enzymatic nucleic acid molecule domain are

sequestered by complementary allosteric effector sequences present in the communication module in the absence of target. Target association with the sensor domain forces an alternative RNA conformation in which the substrate binding elements become available for interaction with cleavage substrate, thus promotion catalysis. **Figure 28** shows a non-limiting example of a nucleic acid sensor molecule that is modulated by a protein target signaling molecule, Erk. In the presence of the target protein (Erk), the nucleic acid sensor molecule (SEQ ID NO. [39]**41**) catalyzes the cleavage of a reporter molecule. Reaction conditions: 100mM KCl, 1mM $MgCl[2]_2$, 10mM Tris 7.5, 10μM ERK protein, 1μM HH ribozyme, Vf=19μl, 34°C for 30 minutes, trace 5' labeled substrate (1μl)."

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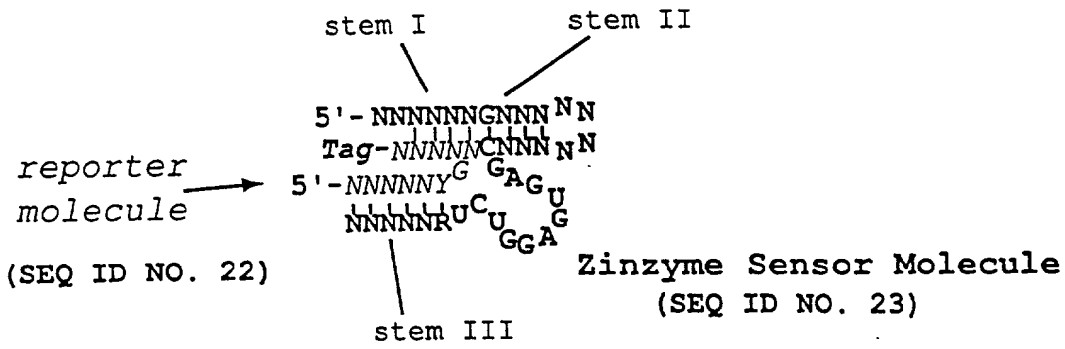
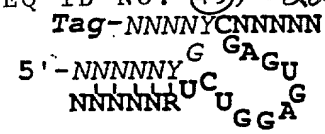
Appendix B

Zinzyme Sensor Molecule for detection of Nucleic Acid



Inactive Zinzyme sensor/ reporter molecule complex

(SEQ ID NO. 49 → 22)

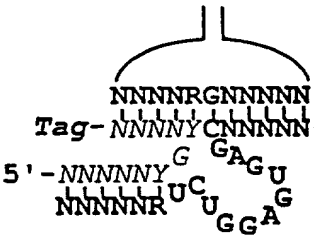


(SEQ ID NO. 24)

Target Signaling Molecule

Target Signaling Molecule

(SEQ ID NO. 25)



(SEQ ID NO. 24)

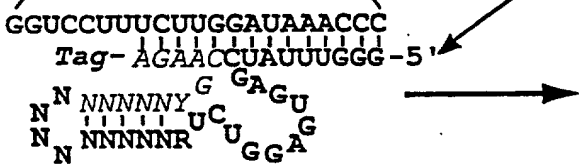
Active Zinzyme sensor/ reporter molecule complex

Stem-loop III of HCV (SEQ ID NO. 26)

(SEQ ID NO 22)

Active HCV Zinzyme sensor/ reporter molecule complex

(SEQ ID NO 27)



catalysis results in release of *Tag-AGAAC for detection*

Zinzyme sensor can be attached to solid support/surface, for example at the 5'-end

FIG. 19

Zinzyme Sensor Molecule for detection of Protein

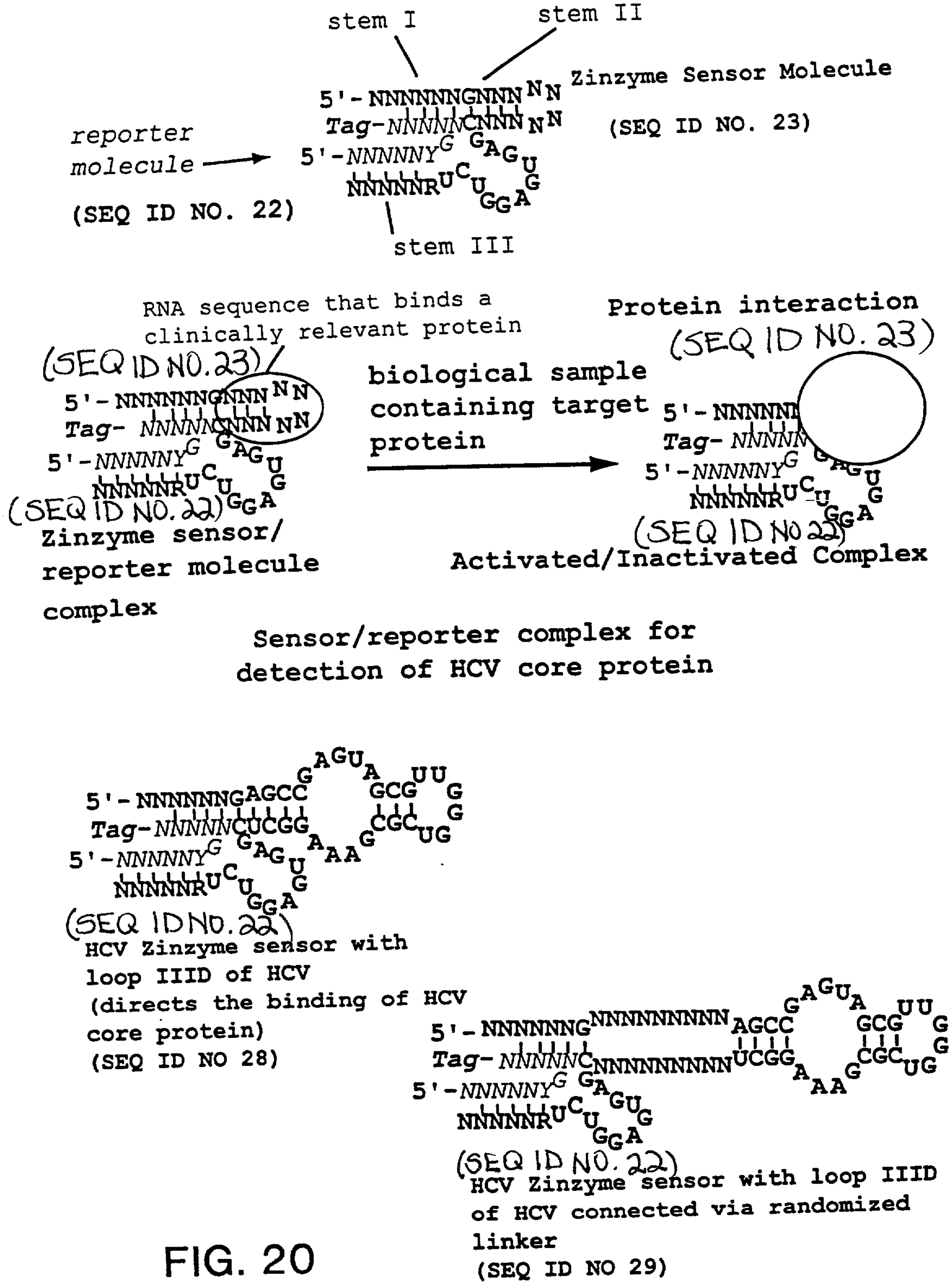

stem I
stem II
5'-NNNNNNGNNNNN
Tag-NNNNNCNNNNN
Zinzyme Sensor Molecule
(SEQ ID NO. 23)
reporter molecule
(SEQ ID NO. 22)
5'-NNNNNY
NNNNNR
stem III
RNA sequence that binds a clinically relevant protein
(SEQ ID NO.23)
biological sample containing target protein
Protein interaction
(SEQ ID NO. 23)
(SEQ ID NO.22)
Zinzyme sensor/ reporter molecule complex
(SEQ ID NO 22)
Activated/Inactivated Complex
Sensor/reporter complex for detection of HCV core protein
5'-NNNNNNGAGCC
Tag-NNNNNCUCGG
(SEQ ID NO.22)
HCV Zinzyme sensor with loop IIID of HCV (directs the binding of HCV core protein)
(SEQ ID NO 28)
5'-NNNNNNGNNNNNNNNNNAGCC
Tag-NNNNNCNNNNNNNNNNUCGG
(SEQ ID NO. 22)
HCV Zinzyme sensor with loop IIID of HCV connected via randomized linker
(SEQ ID NO 29)

FIG. 20

FIG. 26 *Target Inactivation of Zinzyme Sensor Molecule*

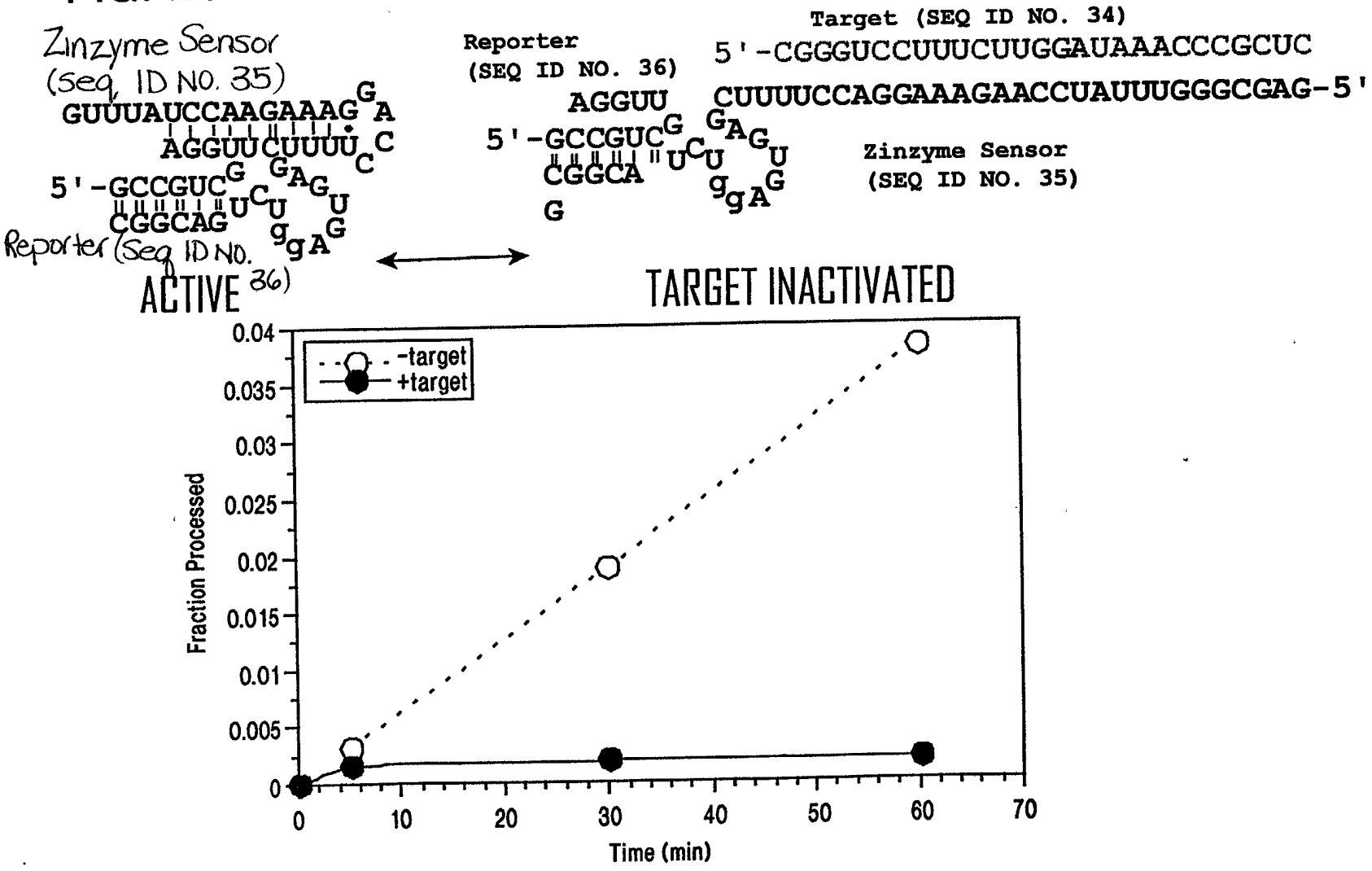


FIG. 27 *Target Activation of Zinzyme Sensor Molecule*

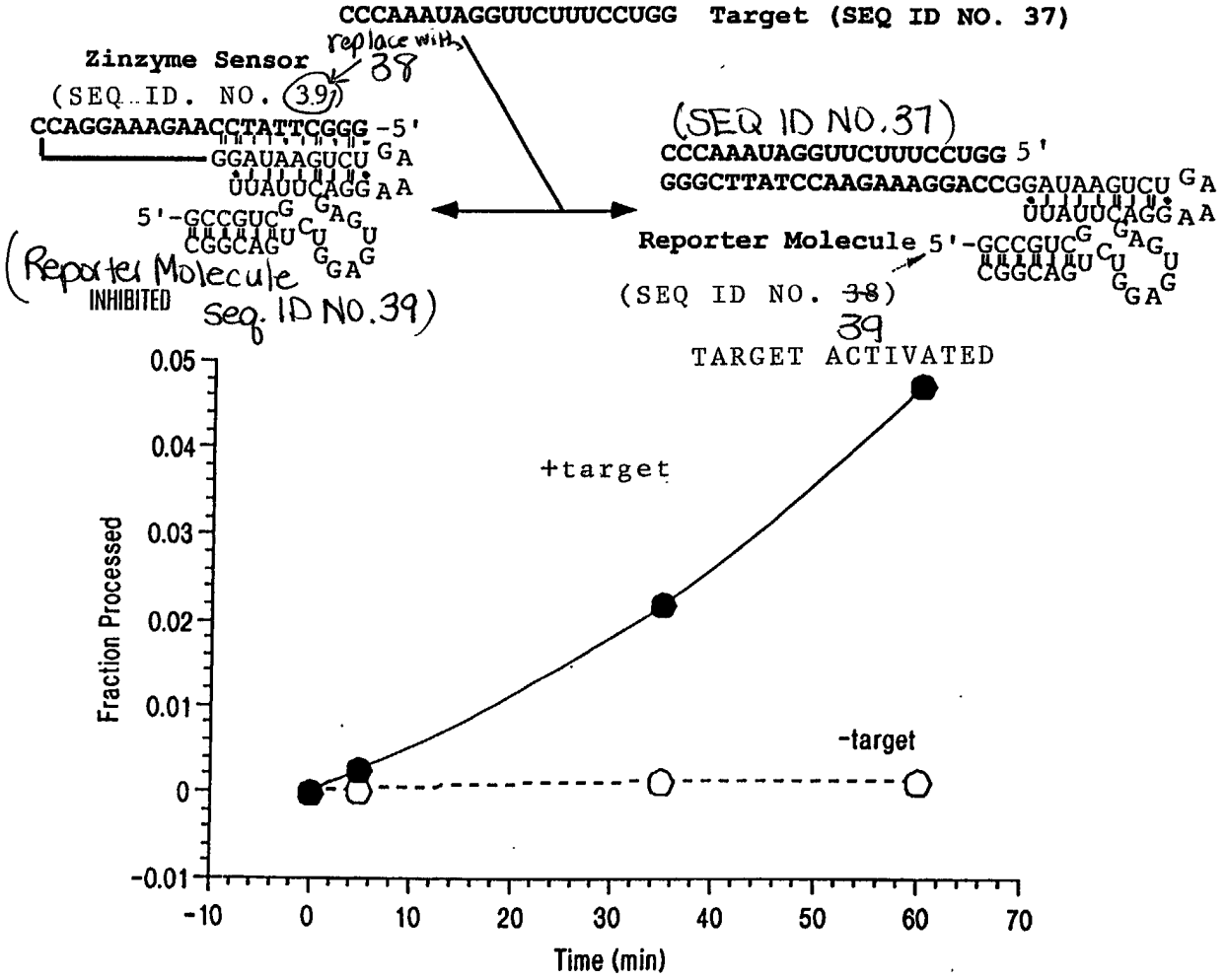


Figure 29: Half-Zinzyme Construct